Frequency and molecular diversity of *Pseudomonas aeruginosa* upon admission and during hospitalization: a prospective epidemiologic study

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Objective: To assess the molecular epidemiology and risk factors for *Pseudomonas aeruginosa* colonization and infection in hospitalized patients.

Methods: In a 1000-bed university hospital, newly admitted patients were assessed prospectively for colonization and infection with *P. aeruginosa*. Anal swabs were obtained upon admission and at discharge. Ribotyping was used for the typing of isolates. Epidemiologic and clinical data were recorded prospectively. Independent risk factors were assessed using multivariate analysis.

Results: The recovery rate of patients with *P. aeruginosa* from anal specimens on admission was 6.7% (42/628). Infection due to *P. aeruginosa* was observed in 20 of 628 (3.2%) patients, of whom 10 (1.6%) were already infected on admission. Independent risk factors for colonization/infection on admission were age, indwelling urinary catheter, the presence of wound and seropositivity for HIV. Independent risk factors for nosocomial infection were anal colonization on admission, alcoholism, indwelling urinary catheter and antibiotic treatment during hospitalization.

Ribotyping revealed that 27 patients were colonized or infected with a unique ribotype, whereas 24 shared one or more ribotypes with other patients. Analysis of epidemiologic and molecular typing data revealed that transmission from patients to patients or from patients to environment was documented on only three occasions.

Conclusions: A great many *P. aeruginosa* strains were isolated from patients and the environment, and most environmental strains were different from those recovered in patients, suggesting a low rate of hospital acquisition of *P. aeruginosa* from the environment. The main risk factors for hospital-acquired infection were detectable colonization on admission, antibiotic treatment and urinary catheter.

Key words: *Pseudomonas aeruginosa*, molecular epidemiology, risk factors

INTRODUCTION

Pseudomonas aeruginosa is an important nosocomial pathogen, being the cause of 10–15% of the nosocomial infections. Moreover, the incidence and relative frequency of hospital-acquired *P. aeruginosa* infection have increased during the last decades [1]. This is partly due to the increased number of patients particularly prone to such infection: immunocompromised patients, and patients with malignancy, cystic fibrosis, burns or traumatic wounds. Infection with *P. aeruginosa* is associated with severe morbidity. Despite antibiotics, the lethality directly or indirectly related to this bacterium is one of the highest among the nosocomial pathogens [2].

P. aeruginosa has multiple environmental reservoirs but can also be part of the endogenous flora of some

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individuals. The environment used to be a major source of nosocomial infections, and epidemics have been linked to contaminated environmental sources such as humidifiers, respiratory equipment, pressure heads, and intravenous solutions. With the advent of effective infection control measures, epidemics have become rare, and endemic nosocomial infections are thought to originate mainly from the endogenous flora of the patients [3]. However, other observations suggest that the hospital environment still plays an important role as a source of endemic infections [4–6]. Thus, although the complex epidemiology of hospital-acquired *P. aeruginosa* infections has long been recognized, our understanding of it is still limited, partly because discriminative molecular typing markers have not been available.

The purpose of the present study was to investigate the molecular epidemiology and risk factors of *P. aeruginosa* colonization and infection in hospitalized patients. Molecular typing of *P. aeruginosa* was performed by ribotyping, using four restriction enzymes, a method which we have shown to be appropriate for epidemiologic studies [7].

MATERIALS AND METHODS

Investigation

The Centre Hospitalier Universitaire Vaudois is a 1000-bed university hospital which was built in the early 1980s. From May to December 1994, patients from 20 units were investigated (365 patients from nine surgical units, and 263 patients from 11 medical units) for clinical and microbiological evidence of *P. aeruginosa* colonization and infection. Each unit was investigated over a period of 2 weeks (small units were grouped and investigated during the same periods). Only the first 10 newly admitted patients (≥18 years old) were included in the study each day (patients transferred from another unit were excluded).

For each patient, anal moist swabs (including the perianal area), as well as swabs of other relevant clinical sites, were performed on admission, during hospitalization when clinically indicated and, whenever possible, at discharge. Environmental sampling included swabs of all moist environmental sites in the vicinity of the patients: toilets, sinks, showers, and baths.

Collection of epidemiologic data

On admission, the following data were recorded: demographic data, information on prior hospitalization(s) during the two previous years, HIV serology, diabetes, alcoholism, neoplasia, presence of indwelling urinary and vascular catheters, presence of wound, immunosuppressive therapy and antibiotics used during the month prior to admission. During hospitalization, the following data were recorded: invasive procedures, presence of wound, presence of indwelling urinary and vascular catheters, immunosuppressive therapy, antibiotic therapy and length of stay.

Laboratory analysis

All swabs were plated within 1 h after sampling onto blood agar plates (incubation at 35°C up to 48 h) and cetrimide-TSA (Pseudosel, Becton Dickinson, Meylan, France) plates (incubation at 42°C up to 48 h). Identification of *P. aeruginosa* was done by standard methods. Ribotyping was performed as already described [7]. Isolates were considered to belong to the same ribotype when sharing the same hybridization patterns for all four restriction enzymes.

Definitions

Patients were considered to be infected with *P. aeruginosa* when the bacterium was isolated from blood or vascular catheters (>15 colonies on semiquantitative culture), from sputum in patients with fever and pulmonary infiltrates, from a draining wound, from urine, or from any normally sterile site. Colonization and/or infection with *P. aeruginosa* were considered to have been hospital acquired if *P. aeruginosa* was not detected in any specimens at the time of admission and if the patient was hospitalized for more than 48 h. Two patients were considered epidemiologically linked if they were hospitalized in the same ward during the same period of time or if they had an identified common source of their infection.

Statistics

Stastistical analysis was performed using SAS software. When risk factors for colonization/infection with *P. aeruginosa* were significant with univariate analysis (data not shown), multivariate analysis was performed.

RESULTS

Colonization and infection on admission

Among 628 patients included in the study, 42 (6.7%) had anal colonization and 10 (1.6%) were infected with *P. aeruginosa* on admission (Figure 1). None had cystic fibrosis. Of the 10 infected patients, four also had positive anal swabs, and three of these had the same ribotype in the infected site and in the anal region. Independent risk factors for being colonized or infected on admission were age (58±18 versus 68±17 years for non-colonized and colonized patients, respectively), presence of an indwelling urinary catheter, presence of a wound and seropositivity for HIV (Table 1).

Nosocomial infection

Among the 567 patients without *P. aeruginosa* infection on admission and hospitalized for more than 48 h, 10 (1.8%) developed a nosocomial infection. Alcoholism, presence of an indwelling urinary catheter, antibiotic therapy and anal colonization on admission were independent risk factors for nosocomial infection (Table 1). Of these 10 patients, two had anal colonization on admission, but only one was colonized with the same ribotype as that found at the infected site. Of the nine patients with nosocomial infection due to a ribotype not present in the anal swab on admission, transmission from other patients was possible on only one occasion (ribotype 26, Figure 2) according to our criteria (hospitalized at the same time in the same unit). It is also of interest to note that among the 20 infected patients (community acquired and nosocomial), five harbored more than one (up to four) different ribotypes.

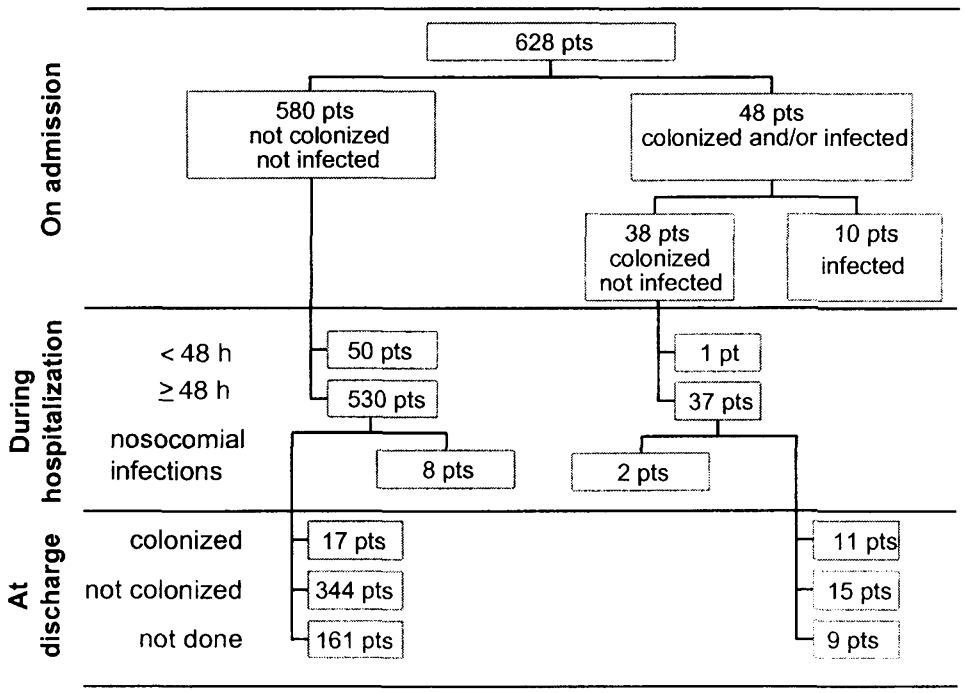

Figure 1 Colonization (anal) and infection with *Pseudomonas aeruginosa* on admission and during hospitalization of the study population.

Table 1 Independent risk factors for colonization/infection with *Pseudomonas aeruginosa* (conditional odds ratio and 95% confidence limits)

Risk factors	(A) Colonization on admission	(B) Infection on admission	(C) Nosocomial infection
Seropositivity for HIV	40.95 (8.58–195)	33.47 (5.06–221)	NS
Indwelling urinary catheter[a]	3.03 (1.12–8.20)	8.01 (1.66–38.7)	7.78 (1.82–33.3)
Presence of wound[a]	3.04 (1.12–8.28)	6.47 (1.34–31.2)	NS
Age (class of 10 years)	1.69 (1.27–2.26)	NS	NS
Antibiotic therapy[a]	NS	NS	10.9 (1.22–98.7)
Alcoholism	NS	NS	8.78 (1.39–55.4)
Anal colonization on admission	–	NS	6.05 (1.00–36.3)

[a] A and B, present on admission. C, present during hospitalization but before *P. aeruginosa* infection.
NS, not significant.

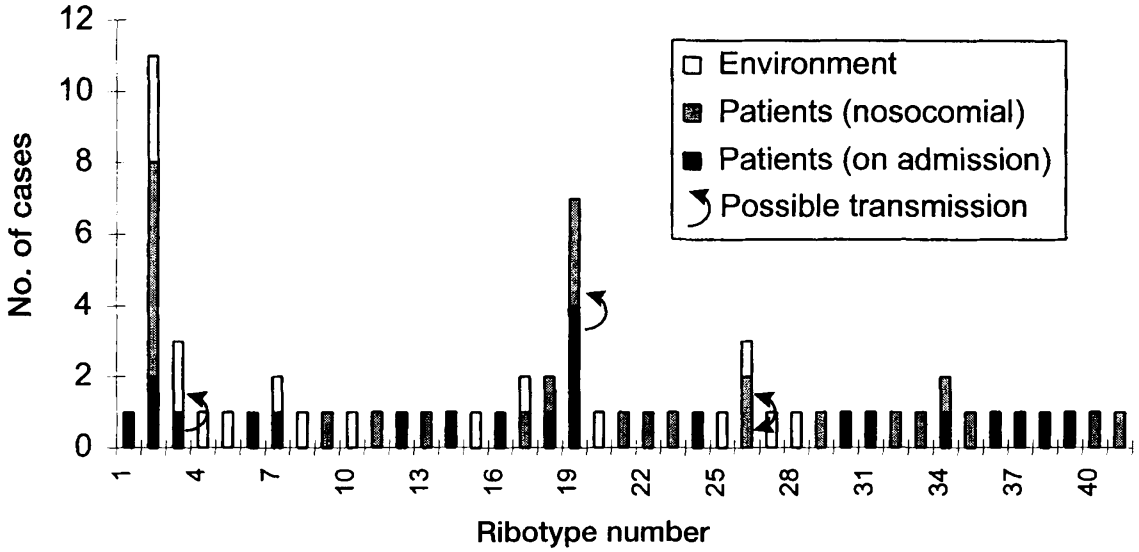

Figure 2 Distribution of *Pseudomonas aeruginosa* ribotypes from patients (one strain of the same ribotype per patient) who were infected and/or colonized on admission or during hospitalization (nosocomial) and from the environment (one strain of the same ribotype per unit for the environment). One hundred and twenty-six isolates were analyzed by ribotyping.

Colonization during hospitalization

Of the 628 patients, 361 cases were (1) hospitalized for more than 48 h, (2) had an anal culture performed both on admission and at discharge, (3) had a negative anal swab on admission and (4) had no infection due to *P. aeruginosa* (Figure 1). Among them, 17 (4.7%) had positive anal swabs at discharge (mean stay in hospital: 15 days), indicating acquisition of *P. aeruginosa* during hospitalization. Risk factors for colonization during hospitalization were neoplasia (conditional odds ratio 3.10; 95% confidence limits 1.10–8.70) and the length of stay in the hospital (conditional odds ratio 1.05; 95% confidence limits: 1.00–1.10).

Ribotyping was performed on isolates of 13 of these 17 patients. Eleven patients harbored a unique ribotype not recovered elsewhere. Two patients shared a ribotype (no. 19 and no. 34, Figure 2) with other patients, but transmission between patients was possible on one occasion (hospitalized during the same period in the same unit).

Environmental sampling

P. aeruginosa was isolated from 34 of 359 (9.5%) environmental samples. All sites showed a low level of contamination (<20 CFU/swab). Twenty-six strains were analyzed and 14 ribotypes were obtained (Figure 2). Twelve ribotypes were unique to a given unit, whereas two were recovered from two and three units, respectively. Only five ribotypes (nos. 2, 3, 7, 17 and 26) were recovered from patients and the environment. On one occasion (ribotype 3), it was clear that the patient was the source of the environmental contamination, since he was admitted for a pneumonia due to this ribotype. In the other situations in which the same ribotype was found in patients and/or in the environment, no epidemiologic link could be found, including for ribotype 2 (Figure 2).

DISCUSSION

As a human pathogen, *P. aeruginosa* is remarkable in its ability to infect certain subpopulations of patients. It has been reported that *P. aeruginosa* predominantly affects hospitalized patients with some predisposing factors, particularly those with burns, cystic fibrosis, traumatic wounds and immunosuppression (especially granulocytopenia). Griffith et al [8] showed that the risk of infection is minimal for non-oncology patients, but may affect as many as 63% of neutropenic patients in certain settings. Moreover, several risk factors are significantly associated with the acquisition of *P. aeruginosa* in hospital, such as use of urinary catheters [8], the length of stay in an intensive care unit (ICU) [9], and the use of antibiotics [10]. In our study, two populations of patients presented risk factors for nosocomial infection and/or acquisition of colonization during hospitalization. The first population was made up of patients already colonized on admission; the risk

factors were HIV infection, advanced age, presence of an indwelling urinary catheter and presence of a wound on admission. Although this population of patients represents only 6% of all admissions, it could play a role as a reservoir of *P. aeruginosa*. The second population comprises patients neither colonized nor infected upon admission. For this population, the risk of acquiring *P. aeruginosa* (colonized or infected) was correlated with the presence of an indwelling urinary catheter, alcoholism, length of stay, neoplasia and antibiotic therapy.

Although colonization by *P. aeruginosa* frequently precedes overt infection, the original source of the organism and the precise mode of transmission are often unclear. While some authors regard patient-to-patient transmission of *P. aeruginosa* via the hands of hospital staff or via fomites as the main routes of transmission, others suggest endogenous colonization as the major source of *P. aeruginosa* infection [10]. Recent epidemiologic studies in intensive care and burn units, using molecular typing methods, have provided arguments for both concepts. In ICUs, Kropec and coworkers [3,11] suggested that endogenous colonization rather than exogenous nosocomial acquisition occurred. However, in burn units, the role of the environment as a source and horizontal transmission have been clearly demonstrated [5,6]. Moreover, Döring et al [4,12] presented indirect evidence that personnel transmitted strains from patient to patient, from patient to sink, and from sink to patient. It should be noted that in this hospital, 90% of all washbasin drains were persistently contaminated with *P. aeruginosa*, whereas in the ICU described by Kropec et al, only a very few environmental sites were found to be contaminated. In our study, *P. aeruginosa* was isolated in less than 10% of environmental samples, despite the fact that sampling was performed in sites where *P. aeruginosa* is usually found (sink, bath, toilet, shower). Moreover, transmission from patient to patient was likely to have occurred only once, and acquisition from the environment was possible for two patients. Altogether, these studies show that the sources and routes of transmission of *P. aeruginosa* vary from one setting to another, and that the importance of the exogenous source may be related to the amount of *P. aeruginosa* present in the environment surrounding the patient. Clearly, all of these factors can be greatly influenced by compliance with good hygiene practices.

The digestive tract is reported to be the major site of *P. aeruginosa* colonization [3,11,13–15]. In our study, anal cultures revealed a colonization rate on admission of 6.7%. This relatively low rate of anal colonization is not different from that found in other studies, in which rates between 2.6% and 24% were reported [8,13]. Other representative site-specific colonization rates were reported to be: skin 0–2%, nasal mucosa 0–3.3% and throat 0–6.6% [13,14]. However, no study has evaluated with molecular methods the variety of the strains colonizing different body sites. This might be of importance for evaluating the hypothesis that gastrointestinal carriage is the major endogenous source for *P. aeruginosa* infection [16]. Indeed, this has been challenged by one study in which no link was found between intestinal colonization and respiratory tract colonization/infection in ICU patients [14]. In our study, only 5 of 20 patients had an infection with the same ribotype as that found in the anal region. The question remains open as to what proportion of *P. aeruginosa* infections could also come from exogenous sources. Indeed, besides the inanimate environment, uncooked vegetables, or even flowers in patients' rooms, may be other sources for endemic *P. aeruginosa* strains. This may account for some of the 17 of 361 patients who acquired colonization during hospitalization in our study. Some of these cases are certainly also due to undetected endogenous colonization on admission (detection problems, overgrowth of *P. aeruginosa* due to antibiotic therapy).

In conclusion, exogenous sources of *P. aeruginosa* in the environment at large may still be important in some hospitals with poor hygiene conditions. In other hospitals, an endogenous origin certainly accounts for a large proportion of cases. However, epidemics due to various exogenous sources such as contaminated respiratory equipment, perfusions or fibroscopes [17] can always occur in any setting. Epidemiologic surveillance combined with molecular typing should help each hospital to better target preventive measures, even though the present study shows that the source of a certain proportion of *P. aeruginosa* infections will remain unclear. Efforts should also be made to reduce known risk factors.

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